ISSN 0325-7541
INFORME BREVE Revista Argentina de Microbiología (2005) 37: 106-108

Isolation of *Mycobacterium gordonae* from poultry slaughterhouse water in São Paulo State, Brazil

K.A. PRINCE[1], A.R. COSTA[1], A.C. MALASPINA[1], A.F. LUÍS[2], C.Q.F. LEITE[1]*

[1]Faculdade de Ciências Farmacêuticas, UNESP, Rodovia Araraquara-Jaú Km 01, C.P 502, CEP 14801-902, Araraquara, SP, Brazil, [2]Faculdade de Ciências Agrárias, UNICASTELO - Campus VIII, Descalvado, SP, Brazil
**Correspondencia. E-mail: leitecqf@fcfar.unesp.br*

SUMMARY

Water samples (24 untreated water, 12 treated water and 24 served water) used in different stages of the slaughter process were examined to identify a possible source of pathogenic mycobacteria. The isolates were identified based on microscopy, morphological and biochemical features, mycolic acid analysis and molecular method - PCR-restriction-enzyme analysis. Eighteen mycobacterial strains were isolated from 60 water samples: 11 from untreated water, 5 from treated water and 2 from served water. All mycobacteria isolated were identified as *Mycobacterium gordonae* and showed the following PRA genotypes: III (27.8%), IV (38.9%) and V (33.3%).

Key words: *Mycobacterium gordonae*, PRA genotypes, poultry slaughterhouse water

RESUMEN

Aislamiento de *Mycobacterium gordonae* de aguas de un abatidero de aves del Estado de São Paulo, Brasil. Muestras de aguas usadas en distintas etapas del proceso de matadero (24 de aguas no tratadas, 12 de aguas tratadas y 24 de procesos industriales) fueron analizadas con el propósito de evaluar posibles presencias de micobacterias patogénicas. Los aislamientos fueron identificados a través de microscopia, aspectos morfológicos y bioquímicos, análisis molecular (PCR-restriction-enzyme analysis) y de ácidos micólicos. Dieciocho aislamientos fueron recuperados de 60 muestras: 11 de aguas no tratadas, 5 de aguas tratadas y 2 de aguas utilizadas en procesos industriales. Todos ellos fueron identificados como *Mycobacterium gordonae* y mostraron los siguientes genotipos de PRA: III (27,8%), IV (38,9%) y V (33,3%).

Palabras clave: *Mycobacterium gordonae*; genotipos de PRA; aguas de abatidero de aves

Water contaminated with pathogenic microorganisms represents health risk. Poultries can also harbor *Mycobacterium avium* and excrete them, acting as carrier of this potentially pathogenic mycobacteria (5). In this sense, it is very important to control the microbiologic quality of water used in poultry slaughterhouses to avoid food contamination (1).

The present study aimed to provide analysis of water used in different stages of slaughter process about the presence of pathogenic mycobacteria.

Samples were collected once a month during a six-month period in ten collection points (1 to 10) and distributed as follow: 24 samples of untreated water (wells - points 1, 2 and 3 and mine - point 4), 12 samples of treated water (1 ppm chlorine - point 5 and 5 ppm chlorine - point 6) and 24 samples of water used for industry (hot water to scald - point 7, washing carcasses - point 8 and cooling tanks - points 9 and 10). Samples were collected in sterilized bottles (1000 ml). The chlorinated samples were collected in bottles with sodium thiosulfate in order to obtain a final concentration of 100 mg/l to neutralize residual chlorine. Mycobacteria dispersed in one liter of water samples were concentrated by two kinds of processes. For the lowest turbid samples (points 1 to 6), the collected water was filtrated through 0.45-µm membrane (Millipore HAWG 04700). For the highest turbid samples (points 7 to 10), the water was centrifuged at 3000g/30min (4). The concentrates were submitted to acid treatment (6). Briefly, the membrane or sediments were treated with 4% H_2SO_4 for 10 minute and neutralized with 30% NaOH. After decontaminated process, 0.2 ml of the sediments were inoculated on 8 slanted tubes of Lowenstein-Jensen (LJ) medium. The cultures were incubated at 30 °C and 37 °C and examined after 2, 7, 14, 21, 30 and 50 days of incubation. To avoid great contamination of the highest turbid samples (points 7, 8, 9 and 10), other strategies and types of media were used besides LJ medium: 1- LJ medium plus ethambutol; 2- Inoculation in Brain Heart Infusion - BHI medium (Difco) at 35 °C for six hours before decontamination process (in order to allow spore germination) (6); and 3- Inoculation in 7H9 medium (Difco) plus mixture of five antibiotics (PANTA, Becton Dickinson) for six days before LJ inoculation. Cultures were checked for purity using Ziehl Neelsen technique. Cultures with acid-fast bacilli were evaluated by colonial morphology and biochemical aspects (3). The identification was com-

pleted with analysis of mycolic acids profiles (8) and molecular characterization by PCR restriction-enzyme analysis (9).

The results about mycobacterial isolation are showed in Table 1. Eighteen isolates were recovered from 60 water samples, 11 from untreated water (well and mine), 5 from treated water (5 ppm chlorine) and 2 from served water samples (washing carcasses and cooling tank at 4 °C). Although poultry is considered to be *M. avium* reservoir (5), this species was not found in the water samples studied. The pre-incubation in 7H9 medium plus PANTA before inoculation in LJ medium was effective reducing contamination and it also enabled the recovery of mycobacteria from samples 8 and 10. Ethambutol incorporated in LJ medium did not avoid growth of proteolytic microorganisms. Similar results were verified with samples incubated in BHI medium before decontamination.

All isolates showed the same smooth, round and pigmented colonial morphology. They were identified as *Mycobacterium gordonae* by biochemical tests and mycolic acid analysis (profile I, III and IV), showing patterns similar to the reference strain *M. gordonae* ATCC 14470. PRA technique confirmed the identification of *M. gordonae* for all isolates and subtyped them into the following PRA genotypes: III (27.8%), IV (38.9%) and V (33.3%) (Table 2). Our PRA results (Table 2) also demonstrated some peculiarities about the three genotypes such as presence of all genotypes in natural water samples, presence of only genotypes IV and V in water with 5 ppm chlorine as well as genotype III in cooling tanks at 4 °C.

In this study, *M. gordonae* was the single species isolated. These microorganisms were present in untreated water and survived through chlorination, they were introduced in the slaughter process and remained at the poultry process until wrapping. *M. gordonae* is ubiquitous in the environment and it shows great resistance to chlorine disinfection (7). Although it is considered to be non-pathogenic, there have been some well-documented cases of

Table 1. Distribution of 18 mycobacterial strains isolated from 60 water samples according to the collect point and incubation media used.

Collect point	Nº of samples	Number of AFB isolated				Total AFB isolated (%)	
		LJ	LJ + EMB	BHI + LJ	7H9 + Panta		
1 (well)	6	3	ND	ND	ND	3	(16,7)
2 (well)	6	1	ND	ND	ND	1	(5,5)
3 (well)	6	5	ND	ND	ND	5	(27,8)
4 (mine)	6	2	ND	ND	ND	2	(11,1)
5 (1 ppm chlorine)	6	-	ND	ND	ND		-
6 (5 ppm chlorine)	6	5	ND	ND	ND	5	(27,8)
7 (hot water to scald)	6	-	-	-	-		-
8 (washing carcasses)	6	-	-	-	1	1	(5,5)
9 (16 °C water)	6	-	-	-	-		-
10 (2 °C water)	6	-	-	-	1	1	(5,5)
Total	60	16	-	-	2	18	

ND: Not determined, AFB: Acid – fast bacilli
Untreated water (1, 2, 3, 4)
Treated water (5, 6)
Served water (7, 8, 9, 10)

Table 2. *Mycobacterium gordonae* PRA genotypes according to the type of water.

PRA pattern	Water type		
	Untreated water	Treated water	Served water
M. gordonae III	4	-	1
M. gordonae IV	4	3	-
M. gordonae V	3	2	1
Total Isolates	11	5	5

infections caused by this organism in patients with underlying conditions, e.g. AIDS or cancer (2). The natural water treatment by chlorination, even with 5 ppm chlorine (point 6), did not eliminate *M. gordonae* but supported the recovery of 27.9% of all the isolates by removing other microorganisms sensitive to this level of chlorine. Our finding warns for the need of constant surveillance by industries of natural water since in an eventual contamination with pathogenic mycobacteria the simple treatment process by chlorination (5 ppm) might not eliminate the pathogen.

REFERENCES

1. American Public Health Association (1992) Standard methods for examination of water and wastewater. 18th edition, APHA, Washington, DC, p. 9-26 -9-61.
2. Bernard E, Michiels JF, Pinier Y, Bourdet JF, Dellamonica P (1992) Disseminated infection as a result of *Mycobacterium gordonae* in a AIDS patient. AIDS. 6: 1217-1218.
3. Brasil (1994) Manual de bacteriologia da tuberculose. 2° edição, Ministério da Saúde, Rio de Janeiro.
4. Dailloux M, Hartemann P, Beurey J (1980) Study on the relationship isolation of mycobacteria and classical micro-biological and chemical indicators of water quality in swimm-ing pools. Zbl. Bakt. Hyg., I. Abt. Orig. B 171: 473-486.
5. Dailloux M, Laurain C, Weber M, Hartemann PH (1999) Water and nontuberculous mycobacteria. Wat. Res. 33: 2219-2228.
6. Falcão DP, Valentini, RS and Leite CQF (1989) Pathogenic or potentially pathogenic bacteria as contaminants of fresh waters from different sources in Araraquara, Brasil. Wat. Res: 27: 1737-1741.
7. Kubálek I, Mysák J (1995) The prevalence of environmental mycobacteria in drinking water supply systems in Olomouc county, north Moravia, Czech Republic, in the period 1984-1989. Centr. Eur. J. Public. Health. 3: 39-41.
8. Leite CQ, De Souza CW, Leite SR (1998) Identification of mycobacteria by thin layer chromatographic analysis of mycolic acids and conventional biochemical method: four years of experience. Mem. Inst. Oswaldo Cruz. 93: 801-805.
9. Telenti A, Marchesi F, Balz M, Bally F, Böttger E.C., Bodmer T (1993) Rapid identification of mycobacteria to the species level by polymerase chain reaction and restriction enzyme analysis. J. Clin. Microbiol. 31: 175-178.

Recibido: 7/2/05 – Aceptado: 23/6/05